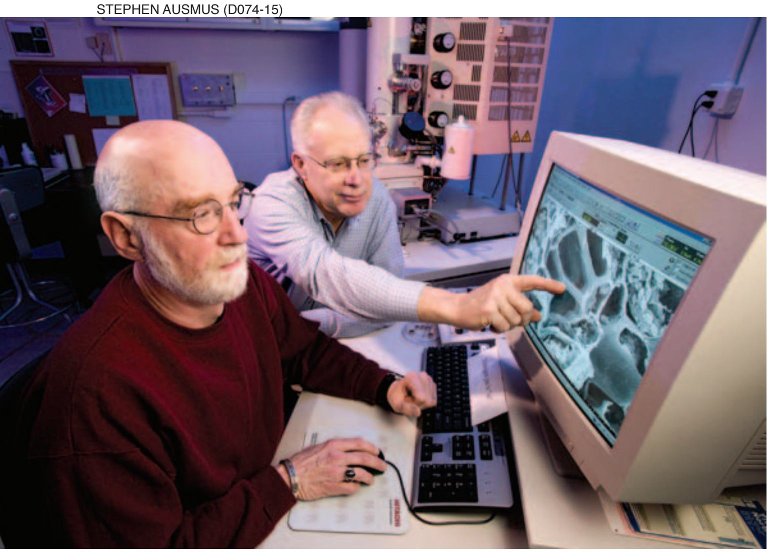
Viral Strike Team Always on Alert

In 2001, a package arrived in the mail, addressed to a little-known team in a laboratory in a small Ohio town. It contained pieces of sweet corn leaves infected by a mystery virus. The viral infection showed up as spots and streaks that ranged in color from pale green to yellow to cream.

The scientific detectives had little to work with. The leaf bits were drying up and falling apart, having been taken off fresh corn plants in Georgia fields several days before. The team had also received a similar package from Arizona, but those leaves showed different symptoms. To identify the suspected viral culprits in both these cases, they needed a lot more infected leaves.

STEPHEN AUSMUS (D074-15)



Research associate Dave Fulton (left), of Ohio State University, and research leader Roy Gingery, of ARS, use a scanning electron microscope to help identify pathogens in corn samples from Serbia.

Fortunately, this group had the tools and expertise to grow their own supply of infected plants for study.

The viral strike team, located at the Ohio State University-Ohio Agricultural Research and Development Center in Wooster, Ohio, serves as the front line for spotting viral attacks on corn worldwide and on corn and soybeans domestically.

The team is part Agricultural Research Service (ARS), part Ohio State University (OSU). Roy Gingery heads the ARS half of the team, which is officially called the Corn and Soybean Research Unit. The other ARS researchers include plant molecular biologist Peg Redinbaugh, molecular geneticist Rouf Mian, and plant pathologist Ray Louie, who is officially retired but works 3 days a week as a research collaborator. Along with their OSU colleagues, these four study interactions among viruses, plants, and insects to develop ways to reduce disease losses.

Technician John Abt says they first tried a "leaf rub" technique with the mystery virus samples as a way to build up a supply of infected plants. They rubbed extracts from the infected leaves onto fresh corn leaves growing in a quarantine greenhouse, but the corn plants didn't get infected.

This didn't particularly surprise Abt or his colleagues; the rubbing technique doesn't work with most viruses. "It may be that this virus needed to reach vascular cells deep within the plant, and simply rubbing the leaf wasn't sufficient to let the virus penetrate deep enough to reach those cells," Abt says.

Another way to create more infected plants for research is to test a variety of insects to see which ones might transmit the viruses. But there wasn't enough plant material to feed insects to allow the tests to start.

Inventing Artificial Insects

That's when the team's vascular puncture inoculation (VPI) technique proved instrumental. Louie has been working on this artificial insect since 1991. The initial prototype was based on a simple concept, and it worked, though it looked pretty primitive. He glued five extremely fine and tiny insect-mounting pins—known as "minutens"—to a flattened copper wire, creating a pin comb. The entire assembly was then inserted into a jeweler's engraving tool.

To use VPI, Abt and Louie ground up infected leaf samples to make a liquid extract, then put a drop of the extract on a presoaked kernel of healthy corn. Next, they pressed the teeth of the pin comb through the extract into the softened kernel, infecting the corn embryo with the help of vibrations from the engraver's tool. This enabled them to implant virus into the corn kernel and grow an infected corn plant.

The VPI method allowed the team to produce enough infected tissue to study the viruses. It eliminated the need to first find and then mass-rear insect carriers, and it required only a few leaf samples to work with.

As a result, the scientists were able to identify what they at first called the "Georgia unknown virus." They later named it "maize fine streak virus." They were surprised to find that the Arizona virus, which they named "maize necrotic streak virus," belonged to the Tombusviridae family, whose members had been known to attack only broadleaf plants such as tomatoes and pepper—not grain crops like corn. Most tombusviruses are spread by soil rather than insects, making them less likely to spread far. "But we have to take it seriously, because we've found that if it were to infect a cornfield, it's unlikely the farmer would be able to harvest a single ear of corn from the field," Redinbaugh says.

Vascular puncture inoculation helps solve plant virus mysteries.

STEPHEN AUSMUS (D078-14)

Technician Tim Mendiola uses "leaf rub inoculation" to transmit bean pod mottle virus to soybeans in a greenhouse at the Ohio Agricultural Research and Development Center in Wooster, Ohio.

She and her team have found one insect carrier of maize fine streak virus, the black-faced leafhopper. They've also mapped the complete genomes of both of the viruses.

Using VPI, the viral strike team has been able to transfer all major corn viruses into corn. They have also used it successfully with other crops, including soybean, wheat, and rice. "This technique gives us the option of mailing infected seeds instead of leaf samples. Seeds withstand shipping better. So far, the method is working very well," Abt says. He has successfully sent infected corn kernels to Wisconsin virus researchers.

If you walked in on Abt and Louie at work, you'd likely see the old and new VPI prototypes at work simultaneously. Abt is infecting a tray of corn kernels with the engraving tool attached to the homemade minuten comb, while Louie uses a greatly updated version. Short, low sound vibrations have replaced the loud, high-pitched buzz of the jeweler's engraver.

Transferring the Technology

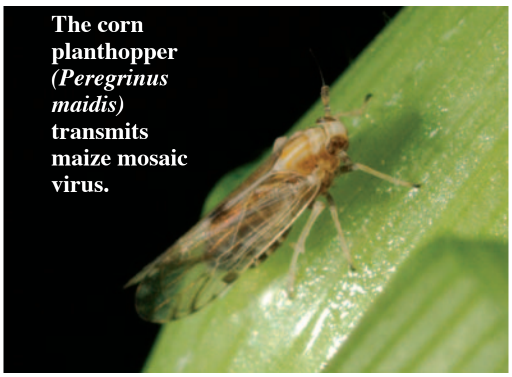
The team has a cooperative research and development agreement with Pioneer Hi-Bred International, Inc., headquartered in Johnston, Iowa, to refine, automate, and optimize today's VPI technique. Pioneer plans to use VPI in breeding programs around the world to screen for resistance to important viral diseases.

While VPI is an important contribution to the cause of identifying and fighting plant viruses, it is by no means the team's only accomplishment.

The scientists supply breeders with viral detection kits. Their corn and soybean genome maps give breeders a road map with markers and clues to help search their own proprietary stock of plant germplasm for viral resistance. Besides identifying new viral diseases and their carriers, the team also seeks to use safe versions of viruses to transfer plant genes into crops to investigate their functions.

Team members travel to foreign countries occasionally. In 2004, Redinbaugh and Gingery, accompanied by OSU maize geneticist Rich Pratt, went to Serbia to help identify a suspected pathogen, perhaps a new virus, attacking corn. While there, they shared the team’s techniques and the VPI method so that Serb scientists could form their own, similar strike team.

ARS CORN AND SOYBEAN RESEARCH UNIT (D083-1)



The corn planthopper *(Peregrinus maidis)* transmits maize mosaic virus.

USDA’s Foreign Agricultural Service requested their services, and they are still working on that identification.

“We have to identify the pathogen first and then the vectors or carriers that spread it. Then we’ll search the corn germplasm collection for genes resistant to it,” Redinbaugh explains.

An important tool for identifying viruses is an electron microscope. The team also makes use of modern imaging tools—such as confocal and scanning electron microscopes—at the Wooster facility’s Molecular and Cellular Imaging Center. “We’re fortunate that all the team members are located nearby,” Redinbaugh says. “Together, we have expertise in several areas, including cloning, genetics, biology, entomology, and virology.”

Answering Viral Threats—Old and New

The team was first formed in the 1960s in response to a dual epidemic of maize chlorotic dwarf virus (MCDV) and a potyvirus, maize dwarf mosaic virus, that devastated Ohio’s cornfields. Today, the Midwest faces potential threats to soybeans because of several factors—excessive rainfall, increased insect numbers from mild winters, widespread use of reduced tillage, less use of crop rotations, entry of the soybean aphid into the United States, and heightened threat of exotic viruses as travel and trade increase, including rising imports of ornamental plants. Ohio, for example, has experienced about every soybean disease imaginable over the past two wet years, 2003 and 2004. Fortunately, a dry fall rescued the 2004 soybean harvest, resulting in record yields.

But in recent years, bean pod mottle virus has emerged as a new pathogen of soybeans in Ohio, along with increased populations of its carrier, the bean leaf beetle. This virus belongs to an important family of viruses, the Comoviridae, and can not only lower yields but also reduce seed quality because of discoloration.

STEPHEN AUSMUS (D075-28)

The black-faced leafhopper *(Graminella nigrifrons)* transmits both maize fine streak virus and maize chlorotic dwarf virus.

STEPHEN AUSMUS (D076-12)

Technician Jane Todd uses an aspirator to collect black-faced leafhoppers *(Graminella nigrifrons)* for transmitting maize fine streak virus.

Soybean aphid populations have been peaking every other year since they were first found in the United States in 2001 in the Great Lakes States, so they may be a problem again in 2005. The researchers are studying the aphids to see whether they’re transmitting any viruses.

Meanwhile, old threats like MCDV cause significant losses in corn in the southeastern United States. The team, led on this project by OSU entomologist Saskia Hogenhout, has produced antibodies that have detected several MCDV proteins in infected plants and insects, including one thought to facilitate transmission. This helper protein serves as a bridge to bind the virus particles to leafhopper mouthparts. Characterization of the interaction between the helper

VPI has enabled the team to transfer all major corn viruses into corn.

protein and the virus particle will help determine how the virus is transmitted from plant to plant during epidemics and may suggest ways to disrupt transmission and, ultimately, prevent epidemics.

Team members Mark Jones, an agronomist, and Bob Anderson, an entomologist (now retired), were largely responsible for identifying the part of the corn genome that holds genes for resistance to MCDV and developing genetic markers for these genes. Redinbaugh says that when she came to Wooster 6 years ago, three accomplishments helped pave the way for her group to identify the resistance genes in corn: The team had found a way to transmit the virus with a high success rate; they'd found corn germplasm with the highest resistance ever—from the Caribbean; and in collaboration with OSU's Rich Pratt, they had designed a way to assign scores to indicate the amount of resistance present in crop germplasm.

Now the current team of ARS and OSU researchers is paving the way for future successes in protecting U.S. crops from viral diseases.—By **Don Comis,** ARS.

This research is part of Plant Diseases, an ARS National Program (#303) described on the World Wide Web at www.nps.ars.usda.gov.

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STEPHEN AUSMUS (D079-6)

Plant molecular biologist Peg Redinbaugh pollinates corn during experiments to map virus resistance.